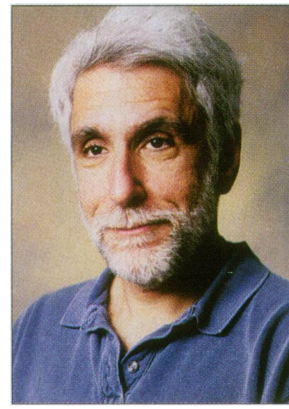
Inflammation may well be only one facet of a time- and dose-dependent continuum of toxicological processes for a specific agent.

Editorial

Inflammation, Tumor Necrosis Factor, and Toxicology



At the recent Society of Toxicology annual meeting in March, Debra Laskin of Rutgers University presented the Burroughs Wellcome Toxicology Scholars Award Lecture titled "Mechanisms of Chemical Toxicity: The Dark Side of the Immune System," which described the role of inflammatory processes in exacerbating hepatotoxicity after use of the common pain reliever acetaminophen.

The role of inflammation in toxicological responses is viewed with considerable interest by many immunologists and toxicologists as more diseases—ranging from arthritis and Alzheimer's to idiopathic pulmonary fibrosis and chronic hepatitis—manifest an inflammatory component that increases, albeit to a varying degrees, disease severity (*1*).

The underlying hypothesis that links these pathologies can be summarized as follows: Initial injury, whether initiated by an infectious, chemical, or environmental agent, produces focal tissue necrosis in a target organ through any one of several toxic mechanisms (e.g., lipid peroxidation, mitochondrial damage). As a result of this damage, tissue-fixed macrophages and circulating monocytes migrate to the damaged site, become activated, and secrete products that cause additional cell damage or induction of inflammatory products. Some of these products are short-lived, such as reactive oxygen species and the nitrogen-centered radical nitric oxide ($NO^{\cdot}$). Other products, such as arachidonic acid and proinflammatory cytokines, regulate the production of additional inflammatory mediators and thus amplify as well as propagate these responses. The overall effect is a chronic inflammatory response resulting in tissue damage that can lead to fibrotic changes.

Although many factors contribute to the inflammatory response, arguably tumor necrosis factor (TNF)α plays the major role in regulating this process. The cellular effects of TNFα include physiologic, cytotoxic, and inflammatory processes (*2,3*). In homeostasis, TNFα influences mitogenesis, differentiation, and immunoregulation while causing apoptotic cell death in neoplastic cell lines. Cytotoxicity by TNFα occurs independently of *de novo* transcription and translation and involves mitochondrial production of oxygen radicals generated primarily at the ubisemiquinone site. This requires ceramide, a sphingolipid generated in cells following stimulation with TNFα, which generates H_2O_2 from the mitochondrial electron transport chain. In contrast to cytotoxicity, TNFα regulates inflammatory processes through the induction of genes that code for mediators [e.g., interleukin (IL)-1, IL-6, IL-8, macrophage inflammatory protein (MIP)-2, granulocyte–macrophage colony-stimulating factor, intracellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1]. In concert, these mediators locally enhance vascular permeability, stimulate the expression of adhesion molecules on endothelial cells, and serve as leukocyte chemoattractants (chemokines).

Once activated neutrophils and circulating monocytes infiltrate a site, they cause additional damage through degranulation and release of neutral proteinase or the generation of superoxide anions (O_2^-) via NADPH-oxidase during the respiratory burst. O_2^-, which itself mediates tissue damage, can be further reduced to other toxic reactive oxygen intermediates, including H_2O_2 and hydroxyl radical ($OH^{\cdot}$) (*4*). The ultimate toxicity of O_2^- may depend on its ability to bring about the reduction of Fe^{+3} to Fe^{+2}, providing for the generation of $OH^{\cdot}$ from H_2O_2, and to interact with $NO^{\cdot}$ to generate $ONOO^{\cdot}$ and subsequently $OH^{\cdot}$. Central to the ability of TNFα to induce inflammatory gene expression is its ability to activate the nuclear transcription factor NF-κB(*5*). NF-κB is a dimer of the Rel family of proteins that becomes activated following dissociation of an inhibitor protein belonging to the IκB family. Cell signaling events that occur following binding of TNFα to cell surface receptors and prior to NF-κB activation are under intense investigation but can involve protein kinases, proteases, protein phosphatases, sphingomyelinases, and phospholipases. Two distinct families of proteins, the TNF receptor-associated factors and the death domain homologs, help coordinate cell- and tissue-specific responses in this complex signaling pathway (*6*).

Several lines of evidence suggest that TNFα mediates organ-specific toxic responses: 1) elevated levels of TNFα can be found in target organs following exposure to certain occupational and environmental agents; 2) administration of TNFα in experimental animals mimics many of the pathophysiological responses associated with the toxic response; 3) inhibition of TNFα, either by administering neutralizing agents or by using TNF transgenic mice (knockouts), prevents many of the pathophysiological responses from occurring; and 4) organs in which these types of responses occur contain tissue-fixed macrophages or other cells that produce high levels of TNFα.

Chronic inflammatory lung diseases, such as idiopathic pulmonary fibrosis, chronic bronchitis, adult respiratory disease syndrome, cystic fibrosis, and some forms of asthma, are associated with elevated TNF responses and neutrophil accumulation in the lung. Increased levels of TNFα have also been found in bronchoalveolar lavage fluid, generated primarily from alveolar macrophages following inhalation of environmental agents associated with pulmonary inflammation (ozone, diesel particles), fibrosis (silica, asbestos), or granulomatous responses (beryllium).

TNFα levels are reduced in the lungs of smokers compared to nonsmokers, which may account for the increased susceptibility of smokers to infections and decreased incidences of some autoimmune and inflammatory diseases. Direct evidence for a role of TNFα in these environmental lung diseases comes from observations that mice pretreated with soluble TNF receptors or neutralizing antibodies to TNFα are resistant to silica or bleomycin-induced pulmonary fibrosis (*7*). Recently our laboratory, in collaboration with Meryl Karol at the University of Pittsburgh, observed that transgenic mice lacking TNF receptors failed to develop any manifestations of TDI asthma including IgE antibodies and inflammation.

A role for TNFα in chemical-induced hepatotoxicity was suggested by studies that demonstrate its involvement in hepatic injury during sepsis and ischaemia reperfusion. TNFα administered to rodents initiates acute phase responses, inflammatory cell infiltration, hyperlipidemia, fibrogenesis, and cholestasis. Subsequent studies indicated that many hepatotoxic chemicals including CCl_4, acetaminophen, $CdCl_2$, alcohol, aflatoxin B_1, and 1,2-dichlorobenzene can be attenuated by agents which deplete neutrophils or block Kupffer cell function, such as gadolinium chloride. Kupffer cells are the resident liver macrophages and the major source of inducible TNFα in the liver. Recently, elevated TNFα levels have been observed in mice exposed to hepatotoxic concentrations of CCl_4, $CdCl_2$, acetaminophen, alcohol, and dimethlylnitrosamine. Furthermore, pretreatment of animals with neutralizing antibodies to TNFα prior to chemical exposure abrogates, albeit to varying degrees, hepatotoxicity associated with a number of these chemical exposures (*8*). Under different conditions, TNFα can promote liver repair following chemical damage by serving as a hepatocyte mitogen (*9*). In this respect, TNFα is required for liver regeneration following partial hepatectomy.

Analogous to its role in maturation of hematopoietic and immunologic cells, TNFα participates in cell apoptosis, differentiation, and proliferation in the central nervous system. Overexpression of TNFα in microglia is associated with autoimmune diseases such as multiple sclerosis and experimental autoimmune encephalomyelitis and has been postulated to be involved in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and AIDS dementia (*10*). Studies on its influence on environmental- or occupational-related neurotoxicity is limited to observations of a rapid and early increase of TNFα message at specific sites in the brain following mechanical damage or exposure to neurotoxic doses of trimethyltin in rodents (*11*).

In the skin, TNFα gene expression occurs in the epidermis within minutes after either physical disruption (UV irradiation, burns, wounding) or application of chemical sensitizers or irritants. The function of TNFα in allergic and irritant contact dermatitis is similar to its role in other organ systems and involves regulation of immune and inflammatory processes. An additional role for TNFα in the skin is to provide a signal for Langerhan's cell migration from the epidermis to the draining lymph node (*12*), an event required for induction of an immune response. Similar to other organ systems, diminished responses to contact allergens and irritants are observed in the skin of mice rendered deficient in TNFα responses either through genetic alterations or administration of neutralizing agents.

In summary, only recently have toxicologists come to appreciate the role inflammation plays in classical toxicological processes. This relationship can be extremely complex, as inflammation may well be only one facet of a time- and dose-dependent continuum of toxicological processes for a specific agent. On the other hand, these mediators are also involved in normal physiological and repair processes. Rendering a host incapable of mounting an inflammatory response would be detrimental. Nonetheless, it may not be long before we administer (as has been indicated for so many other diseases) an aspirin a day as prophylaxis for environmental/occupational maladies.

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